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Determination of antimicrobial resistance to extended-spectrum cephalosporin, quinolones and vancomycin in selected human enteric pathogens from Prince Edward Island, Canada

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Abstract

The aim of this study was to determine the frequency of fecal carriage of vancomycin-resistant *Enterococcus* spp and *Escherichia coli* with reduced susceptibilities to extended-spectrum cephalosporins (ESCs) and quinolones in humans on Prince Edward Island, Canada. Convenience fecal samples from individuals on Prince Edward Island were screened phenotypically using selective culture and genotypically using multiplex polymerase chain reactions to detect *E. coli* and *Enterococcus* spp. resistant to critically important antimicrobials. Twenty-six (5.3%) of 489 individuals had *E. coli* with reduced susceptibility to ESCs. Twenty-five (96.2%) of the 26 isolates harbored *bla*_{TEM}, 18 (69.2%) harbored *bla*_{CMY-2}, 16 (61.5%) harbored *bla*_{CTX-M} groups, 2 (7.7%) harbored *bla*_{SHV} genes. None of the ESC-resistant *E. coli* was positive for carbapenem resistance. Twenty-one (8.3%) of 253 individuals had *E. coli* isolates with reduced quinolone susceptibility. All the 21 isolates were positive for at least one *qnr* gene with 3 (14.3%) isolates positive for *qnrB*, 5 (23.8%) positive for *qnrS*, and 13 (61.9%) were positive for both *qnrB* and *qnrS* genes. All the enterococci isolates were vancomycin-susceptible. Higher susceptibility to the critically important antimicrobials was found in this study. This study can serve as a baseline for future antimicrobial resistance surveillance within this region.

Keywords: Antimicrobial resistance; critically important drugs; *Escherichia coli*; *Enterococcus* spp.; Humans

Introduction

Antimicrobial resistance (AMR) is one of the major public health concerns and urgent challenges of health care systems. Infections and outbreaks caused by antibiotic-resistant bacteria are an established and widely reported problem (Heuer et al. 2009; Marshall and Levy 2011). In some cases, resistance also results in infections with more virulent strains that are difficult to treat, result in prolonged hospitalization, and can be life-threatening (Angulo et al. 2004). This is common in the cases of community-acquired and/or nosocomial infections caused by multi-drug resistant bacteria such as β -lactamase producing *Escherichia coli*, carbapenemase producing *Enterobacteriaceae*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE) (van der Donk et al. 2012; World Health Organization 2014).

Extended-spectrum cephalosporins (3rd and 4th generation cephalosporins), carbapenems, vancomycin, and fluoroquinolones are antimicrobials categorized by the World Health Organization as critically important to human medicine as they are commonly used to treat serious and potentially life-threatening infections caused by antimicrobial-resistant bacteria (World Health Organization 2014). However, increased resistance to these antimicrobials has been reported and is mediated by several different types of AMR genes. β -lactamase genes, such as extended-spectrum β -lactamases (ESBL) and AmpC β -lactamases, are common causes of extended-spectrum cephalosporin (ESC) resistance (Seiffert et al. 2013). Other β -lactamases, such as the carbapenemases including *bla*_{NDM}, *bla*_{KPC}, and *bla*_{VIM}, have been reported in *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter baumannii* and other Gram-negative bacteria, especially members of the *Enterobacteriaceae* family (Nordmann et al. 2011). Although resistance to quinolones can be frequently mediated by chromosomal mutations in DNA gyrase or topoisomerases, plasmid mediated resistance mediated by *qnr* genes such as *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* have been reported from bacteria (such as *E. coli* and *Campylobacter* spp.) of both human and animal origin (Nordmann 2005). Vancomycin resistance in *Enterococcus* spp. are reportedly mediated by *Van* gene clusters including *vanA*, *vanB*, *vanC*, *vanD*, *vanE* and *vanG*, with *vanA* or *vanB* genotypes in *E. faecalis* and *E. faecium* being of most clinical importance (Depardieu et al. 2004).

In their 2013 executive report on antibiotic resistance, the US Center for Disease Control classified carbapenem-resistant *Enterobacteriaceae*, extended-spectrum β -lactamase producing *Enterobacteriaceae*, and vancomycin-resistant *Enterococcus* spp. as urgent and serious threats to

human health (Antibiotic Resistance Threats in the United States, 2013). There is a need for routine monitoring of these bacteria to detect the emergence and changing frequency of these resistant bacteria in community and healthcare settings. Identifying the occurrence and frequency of AMR genes is needed to better understand the epidemiology of these antimicrobial resistant bacteria. Currently, information on the frequency and distribution of these important antibiotic-resistant bacteria and their resistant genes in humans from Prince Edward Island (PEI) is limited. The primary objective of this study was to determine the frequency of fecal carriage of vancomycin-resistant *Enterococcus* spp. (VRE), ESC- resistant *E. coli*, quinolone- resistant *E. coli*, and carbapenem-resistant *E. coli* (CRE) in patients from PEI. The second objective was to determine the antimicrobial minimum inhibitory concentration (MIC) profile and the antimicrobial resistance genes present on a subset of the bacterial isolates.

Materials and methods

Human fecal sample collection

This study was approved by the Research Ethics Boards of the University of Prince Edward Island and the Province of Prince Edward Island. Four hundred and eighty-nine human fecal samples were collected from samples submitted for routine *Helicobacter pylori* screening at the Queen Elizabeth Hospital (QEH) Diagnostic Laboratory in Charlottetown, PEI, Canada from August to December 2016. The 6-character postal code, anonymous patient ID, and the date of sample collection were the only epidemiological data collected for analysis. These samples were stored refrigerated in individual sterile containers and were collected twice a week by the QEH laboratory technicians. Samples were transported to the research laboratory at the University of Prince Edward Island, Charlottetown Canada in a cooler with ice and were processed on the day of receipt.

Fecal sample processing

In the research laboratory, 3 to 5 grams of feces were used for bacterial culture. Feces was mixed with 9 times its weight of buffered peptone water (BPW) (Difco™ Buffered peptone water, Becton, Dickson and Company, USA). For *E. coli*, half of the BPW aliquot was mixed 1:1 with double-strength *E. coli* enrichment broth and incubated at 44°C for 18-24 hours. For *Enterococcus* spp., the remaining half of the BPW aliquot was also mixed 1:1 with double-strength Enterococcosel broth and incubated at 35°C for 18-24 hours. Selective culture plates

were used to recover *Enterococcus* spp. with reduced susceptibility to vancomycin, and *E. coli* with reduced susceptibilities to ESC (sESC-R *E. coli*) and quinolones (sQNR-R *E. coli*).

Isolation and identification of sESC-R *E. coli* and sQNR-R *E. coli*

All fecal samples (n=489) were used for the isolation and identification of sESC-R *E. coli* and 253 were used for the isolation and identification of sQNR-R *E. coli*. A tryptic soy agar (DifcoTM Tryptic soy agar, Becton, Dickson and Company, USA) plate with 5% sheep blood containing vancomycin (6 µg/mL), ceftazidime (2 µg/mL), amphotericin B (2 µg/mL) and clindamycin (1 µg/mL) (VACC) was used for selective culture of sESC-R *E. coli* (Singh et al. 2012). MacConkey agar (DifcoTM MacConkey agar, Becton, Dickson and Company, USA) supplemented with 0.5 µg/mL ciprofloxacin (MAC-C) was used for the isolation of sQNR-R *E. coli*. VACC and MAC-C plates were inoculated with 50 µL of *E. coli* enrichment broth and then incubated at 35°C for 18-24 hours. If no presumptive *E. coli* colonies were observed, VACC and MAC-C plates were incubated for an additional 18-24 hours. Presumptive *E. coli* were sub-cultured to eosin methylene blue agar and typical colonies were purified on a tryptic soy agar plate supplemented with 5% sheep blood (TSA-B). *E. coli* were further identified by lactose-fermentation, utilization of citrate, and indole test. All the *E. coli* isolates recovered from VACC plates were further screened for carbapenem resistance using the Carba NP (Carbapenemase Nordmann-Poirel) test (Biomérieux, USA) according to the manufacturer's instruction and as reported by (Nordmann et al. 2012). All the isolates were frozen in Brucella broth (DifcoTM Brucella broth, Becton, Dickson and Company, USA) with 15% glycerol at -80°C for further laboratory analysis.

Isolation and identification of vancomycin-resistant *Enterococcus* spp

VACC agar was inoculated with 50 µL of Enterococcosel enrichment broth and incubated at 35±2°C for 18-24 hours. If no presumptive *Enterococcus* colonies were observed, the VACC plates were incubated for an additional 18-24 hours. Presumptive *Enterococcus* spp. from the VACC plates were sub-cultured to Enterococcosel agar (EA) plates (BBLTM EnterococcoselTM Agar, Becton, Dickson and Company, USA). Esculin-positive colonies were purified on TSA-B and tested for catalase. *Enterococcus* isolates were further identified to the species level by use of Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass

Spectrometry (MALDI-TOF) using the Bruker microflex LT and BioTyper RTC 3. All isolates were frozen in Brucella broth with 15% glycerol at -80°C for further laboratory analysis.

Antimicrobial susceptibility testing

For all recovered sESC-R *E. coli* isolates, antimicrobial susceptibility testing was performed using the Sensititre™ broth microdilution method to determine the minimum inhibitory concentrations (MICs) to a panel of antimicrobials. The testing was carried out according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015). The CMV2AGNF (Sensititre™, Trek™ Diagnostic Systems, Westlake, Ohio) susceptibility plate of the National Antimicrobial Monitoring System (NARMS) containing 15 antimicrobials was used in this study, however, azithromycin was excluded because of the intrinsic resistance in *E. coli* (Kozak et al. 2009).

The following antimicrobial agents were tested with the resistance breakpoints presented in parentheses (CLSI, 2015): ampicillin (≥ 32 µg/ml), amoxicillin-clavulanic acid ($\geq 32/16$ µg/ml), chloramphenicol (≥ 32 µg/ml), ceftriaxone (≥ 4 µg/ml), ceftiofur (≥ 8 µg/ml), ciprofloxacin (≥ 4 µg/ml), ceftiofur (≥ 32 µg/ml), gentamicin (≥ 16 µg/ml), kanamycin (≥ 64 µg/ml), nalidixic acid (≥ 32 µg/ml), streptomycin (≥ 64 µg/ml), sulfisoxazole (≥ 512 µg/ml), trimethoprim-sulfamethoxazole ($\geq 4/76$ µg/ml), and tetracycline (≥ 16 µg/ml). Multi-drug resistance was based on the World Health Organization's definition of resistance to at least one antimicrobial each in ≥ 3 antimicrobial categories (Magiorakos et al. 2012). Based on MIC testing, isolates showing resistance to either/or both ceftriaxone and ceftiofur were considered as ESBL phenotypes and ESC-resistant, while isolates not showing resistant to both antimicrobials were considered as ESC-susceptible. Resistance to ceftiofur, amoxicillin-clavulanate in addition to resistance to either/or both ceftriaxone and ceftiofur were considered as AmpC phenotypes.

For the *Enterococcus faecalis* and *Enterococcus faecium* isolates, antimicrobial susceptibility testing was carried out to determine the MIC to vancomycin by using vancomycin MIC test strips (Liofilchem®, Italy) with MIC values from 0.016-256 µg/ml (breakpoint of ≥ 32 µg/ml). The testing was carried out according to the Clinical and Laboratory Standards Institute guidelines and protocols (CLSI, 2015).

Molecular characterization of antimicrobial resistance genes

Genomic DNA of all the sESC-R *E. coli* and suspect vancomycin resistant *Enterococcus* isolates was extracted using the InstaGene™ Matrix following manufacturer's guidelines (Bio-Rad, Montreal Canada). For all multiplex PCRs, a Qiagen multiplex PCR kit (Qiagen, Mississauga, Ontario, Canada) with 1X Qiagen multiplex PCR master mixture, 1X Q-solution, and molecular grade water, together with 1X consensus primer pair mixture as well as 2 µl of the isolate's DNA template were included in the final 25 µl mixtures according to the manufacturer's instructions. Positive and negative controls were included in every multiplex PCR. Amplification was carried out in the PCR thermocycler (Techne TC-412 Thermo Fisher Scientific, Mississauga Ontario). DNA fragments were analyzed by electrophoresis on a 1.2% agarose gel at 100 V for 1 hour in 1X Tris-boric acid-EDTA stained with 1 µg/mL ethidium bromide.

For sESC-R *E. coli* isolates, two multiplex PCRs were performed for molecular characterization of β-lactamase genes. The first multiplex PCR for the detection of *bla*_{CMY-2}, *bla*_{SHV}, and *bla*_{TEM} was carried out using the consensus primers and protocol (Table 1) previously described by (Kozak et al. 2009). While the second multiplex PCR was carried out to determine the different *bla*_{CTX-M} groups (group 1, 2 and 9) using the consensus primers and protocol (Table 1) previously described by (Dallenne et al. 2010). Amplification was carried out using the following PCR conditions for the first multiplex PCR: one cycle consisting of 15 min at 94°C; 30 cycles consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and one cycle of final extension consisting of 10 min at 72°C. While an initial denaturation at 94°C for 10 min; 30 cycles of 94°C for 40 s, 60°C for 40 s and 72°C for 1 min; and a final elongation step at 72°C for 7 min was used for the second multiplex PCR.

For sQNR-R *E. coli* isolates amplification of plasmid-mediated quinolone resistance (PMQR) genes including *qnrB* group, and *qnrS* group genes was performed using the published consensus primers and protocol (Table 1) previously described by (Cattoir et al. 2007). Amplification was carried out using the following PCR conditions: 10 min at 95°C and 35 cycles of amplification consisting of 1 min at 95°C, 1 min at 54°C and 1 min at 72°C and 10 min at 72°C for the final extension.

Detection of *VanA* and *VanB* were done in a multiplex PCR reaction using the published primer sequences and protocol (Table 1) previously described by (Depardieu et al. 2004). Amplification was carried out using the following PCR conditions: 3 minutes at 94°C and 30

cycles of amplification consisting of 1 minute at 94°C, 1 minute at 54°C and 1 minute at 72°C and 7 minutes at 72°C for the final extension.

Distribution of the resistant enteric bacteria based on postal code

The relationship between the resistant bacteria and postal codes was examined. The distribution of antimicrobial resistant bacteria based on where the patients originated from was examined to determine the difference in the frequency of resistant bacteria between the urban and rural communities. Comparison between urban and rural sources was achieved using Chi-square test.

Results

Extended-spectrum cephalosporin resistance in generic *Escherichia coli* isolates

Twenty-six (5.3%) of the human fecal samples were positive for sESC-R *E. coli* of which 14 (53.9%) isolates showed antimicrobial resistance profiles consistent with ESBL phenotypes, 9 (34.6%) isolates had antimicrobial resistance profiles consistent with AmpC phenotypes and 3 (11.5%) did not demonstrate phenotypic ESC resistance on MIC testing. Resistance to ampicillin, ceftriaxone, ceftiofur, sulfisoxazole, and tetracycline were common (Table 2), with multi-drug resistance observed in 22 (84.6%) of the 26 sESC-R *E. coli* isolates (Table 3). All 26 sESC-R *E. coli* isolates were positive for at least one β -lactamase gene. The ESC-resistant gene profile was as follows: 25 (96.2%) of the isolates harbored *bla*_{TEM}, 18 (69.2%) of the isolates harbored *bla*_{CMY-2}, 16 (61.5%) of the isolates harbored *bla*_{CTX-M} groups (*bla*_{CTXM-1} = 9 isolates; *bla*_{CTXM-9} = 5 isolates; and *bla*_{CTXM-2} = 2 isolates), and 2 (7.7%) isolates harbored *bla*_{SHV} resistance genes. Twenty-four (92.3%) isolates harbored 2 or more β -lactamase resistance genes (Table 4). The three sESC-R *E. coli* isolated using selective culture with an ESC-susceptible phenotype on MIC testing were *bla*_{TEM} and *bla*_{CMY-2} positive and two of these isolates harbored either *bla*_{SHV} or *bla*_{CTXM-9}. All sESC-R *E. coli* isolates were negative for carbapenem resistance.

Quinolone resistance in generic *Escherichia coli* isolates

Of the 253 fecal samples cultured, 21 (8.3%) were sQNR-R *E. coli* positive. Based on MIC testing, 15 (71.4%) of the 21 isolates were resistant to nalidixic acid while 12 (57.1%) were resistant to ciprofloxacin. All ciprofloxacin-resistant *E. coli* were nalidixic acid resistant. Resistance to ampicillin, tetracycline, sulfisoxazole and nalidixic acid was common (Table 5)

and 18 (85.7%) of the 21 sQNR-R *E. coli* isolates were multi-drug resistant (Table 6). Seven of the 253 fecal samples cultured for sQNR-R *E. coli* were also sESC-R *E. coli* and phenotypically all had ESBL profiles. All 21 sQNR-R *E. coli* isolates were positive for at least one *qnr* gene. Three (14.3%) of the isolates were positive for *qnrB* gene alone, 5 (23.8%) were positive for *qnrS* gene alone and 13 (61.9%) were positive for both *qnrB* and *qnrS* genes. Of the 13 *qnrB* and *qnrS* positive isolates, 10 were both ciprofloxacin and nalidixic acid resistant on MIC testing.

Vancomycin resistance in generic *Enterococcus* spp. isolates

Enterococcus spp. with reduced susceptibility to vancomycin were isolated in 68 (13.9%) of the patients. Only 10 (14.7%) of the isolates were *E. faecalis* and 2 (2.9%) were *E. faecium*. Other *Enterococcus* spp isolated were *E. gallinarum* (52 isolates) and *E. casseliflavus* (4 isolates). All *E. faecalis* and *E. faecium* suspect vancomycin resistant isolates had MIC values between 0.5-4 µg/ml and, therefore, were considered susceptible to vancomycin. None of these isolates were positive for either *vanA* or *vanB*.

Household and community distribution of the resistant enteric bacteria

Two sESC-resistant *E. coli* isolates that produced *bla*_{TEM} and *bla*_{CMY-2} were within the same postal code. Similarly, another postal code was associated with two sESC-resistant *E. coli* isolates that produced both *bla*_{TEM} and *bla*_{CTXM-1}. A third postal code was associated with 2 *bla*_{TEM} producing *E. coli*. Every other postal code was associated with only one sESC-resistant *E. coli* isolates. There was no significant difference (P=0.096) in the frequency of sESC-resistant *E. coli* isolates between the urban (61.5%) and rural municipalities (38.5%). Similarly, different ESC-resistance genes were not associated with particular municipalities.

Three patients that were positive for *qnrB* producing *E. coli* were associated with the same postal code, while another postal code was associated with two quinolone-resistant *E. coli* isolates that were both *qnrB* and *qnrS* positive. Similarly, two *Enterococcus faecalis* isolates were also associated with the same postal code. There was significant difference (P<0.031) in the frequency of quinolone-resistant *E. coli* isolates between the urban (66.7%) and rural municipalities (33.2%). However, there was no association between the different PMQR genes and the municipalities.

Discussion and conclusion

Monitoring and surveillance of antimicrobial resistance is an important step in elucidating the extent of the threat posed by antimicrobial resistant bacteria (Antibiotic Resistance Threats in the United States, 2013). This allows for a better understanding and greater knowledge of the emergence of bacterial pathogens within a region. The frequencies of fecal carriage of ESC-resistant and quinolone-resistant *E. coli* were <10% and carbapenem resistant *E. coli* as well as, vancomycin resistant *Enterococcus faecalis* or *Enterococcus faecium* were not found in this study. These findings are consistent with susceptibilities of clinical isolates from both community and long-term care centers on PEI (Health PEI, Unpublished data). This suggests a low level risk of these resistant bacteria within the province of PEI. These results are encouraging considering (i) these critically important antimicrobials are among the most commonly used antimicrobials in human medicine in Canada, especially for the treatment of extra intestinal *E. coli* infections (Ebrahim et al. 2016), and (ii) infections with carbapenemase-producing *Enterobacteriaceae* (CPE) and VRE have been reported from other parts of Canada (McCracken et al. 2013; Ebrahim et al. 2016).

In this study, patients shedding the ESC and/or quinolone-resistant *E. coli* may have acquired the isolates via food-exposure. The fecal samples in this study were collected from the patients being screened for *Helicobacter pylori*, which are likely outpatients and not hospitalized patients. Also, a few patients with the same postal code shared similar resistance genes, which suggest the possibility of a common source of exposures. A similar study in patients at a tertiary care centre in Ohio, USA has also suggested food-borne transmission as a source of β -lactamase producing *E. coli* in humans (Landers et al. 2016). Also, reports from the Netherlands have shown a phylogenetic similarity in antimicrobial resistance genes originating from meat and human enteric isolates (Johnson et al., 2007; Overdevest 2011). Alternative transmission routes including health-care related exposure have been documented in other studies (Johnson et al. 2008; Leistner et al. 2013).

Detection of β -lactamase resistance genes and PMQR genes in the *E. coli* isolates suggests that these bacteria could colonize the human gastrointestinal tract and can serve as a reservoir of important AMR genes. These genes can be transferred through mobile genetic elements to other commensal flora and/or to more pathogenic resistant strains (Hannah et al. 2009), and in some cases cause multi-drug resistant extra-intestinal infections (Johnson et al. 2001). The *bla*_{TEM} and *bla*_{CMY-2} genes were the most prevalent β -lactamases in this study, which are similar to the

genotypes present in dairy calves (Awosile et al. 2018) and in retail meats within Atlantic Canada (Forward et al. 2004) as well as other parts of North America (Daniels et al. 2007; Martin et al. 2012). Previous studies from the Netherlands have reported on the genetic similarities of AMR resistance genes in Dutch patients, poultry and retail meat (Leverstein-van Hall et al. 2011; Overdevest et al. 2011). The *bla*_{CTX-M} resistance group of genes are known to be the dominant ESBL type in Europe in generic *E. coli* of human enteric origin (Denisuik et al. 2013), however the frequency of carriage of *bla*_{CTX-M} in *E. coli* was lower in this study compared with the carriage of *bla*_{TEM}. The predominant *bla*_{CTX-M} group in generic *E. coli* colonizing human enteric flora in this study was *bla*_{CTX-M} group 1, which is similar to other studies (Valenza et al. 2014; Landers et al. 2016).

A relatively high frequency (8.3%) of *qnr* positive isolates was observed in this study, which differs from previous studies in Canada that found lower frequencies (Pitout et al. 2008; Peirano et al. 2012). However this study utilized selective culture media containing ciprofloxacin for culture, whereas most other studies did not utilize selective culture media. Pitout et al. (2008) reported a low-level frequency of detection (0.8%) for *qnrS*, while Peirano et al. (2012) reported no detection of any *qnr* genes in their study conducted in Calgary, AB. Interestingly, *qnrA*, *qnrB* and *qnrS* PMQR genes have been reported recently from human *Salmonella* isolates from Alberta (Kim et al. 2016). Plasmid-mediated quinolone resistance genes are known to confer reduced susceptibilities to quinolones at a level below the susceptibility breakpoints (Allou et al. 2009). PMQR genes were detected from all sQNR-R *E. coli* isolated from selective media, including the quinolone MIC-susceptible isolates in our study. This is unsurprising as these genes rarely initiate large increases in MICs leading to quinolone resistance except in mutant strains, which may occur following therapeutic fluoroquinolone exposure or in porin-deficient pathogens (Robicsek et al. 2006).

Enterococcus gallinarum was the predominant *Enterococcus* spp. in this study accounting for over 70% of the enterococci isolated. Although this species produces low-level vancomycin and constitutive *vanC* type glycopeptide resistance, their clinical importance is questionable, as they are rarely recovered from clinical cases (Reid et al. 2001). Vancomycin-resistant *Enterococcus faecium* has been reported to be the most prevalent vancomycin-resistant *Enterococcus* spp., accounting for the majority of the cases of VRE infections of both healthcare-associated and community-acquired origin (Depardieu et al. 2004). In Canada, similar findings

have been reported from clinical isolates from a Canadian hospital surveillance program (Zhanel et al. 2013). Interestingly VRE were not detected in our study from this region. Previous studies have reported that the frequency of VRE genotypes within eastern Canada is low, even though they were first described in Canadian hospitals over two decades ago (McCracken et al. 2013). This study suggests that vancomycin-resistant *Enterococcus* spp. is in low frequency among humans in PEI and perhaps elsewhere in Atlantic Canada.

The limitations and bias of this study should be considered when interpreting the results as well as in making comparison with other studies. Patient information such as recent hospitalization, previous or current antimicrobial treatments, or nursing home admission were not available in this study. Convenience fecal samples from outpatient individuals being tested for *Helicobacter pylori* infection were used in this study, which may bias our inference in comparison with the general population. We do not know the age of these patients as convenience sampling may have biased our collection to mainly adults which may underestimate the true frequencies of these resistant-bacteria. Representative sampling cutting across all age groups may give a better estimate of the true frequencies of these resistant-bacteria. The selective culture plates used in this study may have contributed to a higher rate of β -lactamase genes and PMQR genes being detected compared with other studies.

In conclusion, the frequency of fecal carriage of ESC-resistant and quinolone-resistant *E. coli* were low, and carbapenem resistance in *E. coli*, as well as VRE, were not found in this study. *Bla*_{TEM} and *bla*_{CMY-2} were the predominant β -lactamase resistance genes harbored by *E. coli* from human fecal samples in this study. Higher frequencies of PMQR genes were observed in this study with *qnrB* and *qnrS* being the most common. This study can serve as a baseline source for future antimicrobial resistance surveillance within this region.

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Table 1: Multiplex PCR primer sequences used for the detection of antimicrobial resistance genes in human enteric *E.coli* and enterococci

AMR genes	Primers	Primer sequence	Final primer concentration (μM)	Amplicon size (bp)	Reference
TEM	F	TTAACTGGCGAACTACTTAC	0.2	247	Kozak et al., 2009
	R	GTCTATTTCGTTTCATCCATA	0.2		
SHV	F	AGGATTGACTGCCTTTTTTG	0.4	393	
	R	ATTTGCTGATTTCGCTCG	0.4		
CMY-2	F	GACAGCCTCTTTCTCCACA	0.2	1000	
	R	TGGACACGAAGGCTACGTA	0.2		
CTXM-1 group	F	TTAGGAARTGTGCCGCTGYA ^{ab}	0.4	688	Dallenne et al., 2010
	R	CGATATCGTTGGTGGTRCCAT ^b	0.2		
CTXM-2 group	F	CGTTAACGGCACGATGAC	0.2	404	
	R	CGATATCGTTGGTGGTRCCAT ^b	0.2		
CTXM-9 group	F	TCAAGCCTGCCGATCTGGT	0.4	561	
	R	TGATTCTCGCCGCTGAAG	0.4		
<i>qnrB</i> group	F	GGMATHGAAATTCGCCACTG ^c	0.2	264	Cattoir et al., 2007
	R	TTTGCYGY ^d CGCCAGTCGAA ^a	0.2		
<i>qnrS</i> group	F	GCAAGTTCATTGAACAGGGT	0.2	428	
	R	TCTAAACCGTCGAGTTCGGCG	0.2		
<i>VanA</i>	F	GGGAAAACGACAATTGC	0.4	732	Depardieu et al., 2004
	R	GTACAATGCGGCCGTTA	0.4		
<i>VanB</i>	F	ACGGAATGGGAAGCCGA	0.4	647	
	R	TGCACCCGATTTCGTTC	0.4		

^aY=T or C; ^bR=A or G; ^cM = A or C; ^dH = A or C or T

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Table 2: Distribution of minimum inhibitory concentrations for sESC-R *Escherichia coli* ($n=26$) recovered from human fecal samples from Prince Edward Island

Antimicrobials	Distribution of MIC (μg/mL)																			
	Range	MIC ₅₀	MIC ₉₀	% R ^c	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Amoxicillin/clavulanate	1 – 32	16	>32	46.2									3.9 ^d	26.9	23.1	3.9	42.3			
Ampicillin	1 – 32	>32	>32	100.0													100.0			
Cefoxitin	0.5 – 32	16	>32	46.2								3.9	26.9	15.4	7.7	11.5	34.6			
Ceftiofur ^a	0.12 – 8	16	16	84.6								11.5	3.9	11.5	73.1					
Ceftriaxone	0.25 – 64	32	>64	88.5						3.9	7.7		3.9	11.5	19.2	7.7	11.5	34.6		
Chloramphenicol	2 – 32	8	8	3.9									23.1	69.1	3.9		3.9			
Ciprofloxacin	0.015 – 4	0.06	>4	23.1	38.5	7.7	3.9	3.9	3.9	15.4	3.9			23.1						
Gentamicin	0.25 – 16	1	>16	23.1					3.9	30.8	38.3		3.9			23.1				
Kanamycin	8 – 64	8	8	7.7										76.8	7.7	7.7	3.9	3.9		
Nalidixic acid	0.5 – 32	4	>32	30.8							11.5	26.9	19.2	11.5		7.7	23.1			
Streptomycin ^b	32 – 64	32	>64	50.0												50.0		50.0		
Sulfisoxazole	16 – 512	>256	>256	61.5											19.2	19.2				61.5
Tetracycline	4 – 32	>32	>32	61.5									38.3		3.9	3.9	53.9			
Trimethoprim/ Sulfamethoxazole	0.12 – 4	0.5	>4	50.0				46.1		3.9				50.0						

^a CLSI M31–A2

^bNo Clinical and Laboratory Standards Institute interpretive criteria for *Enterobacteriaceae* were available, breakpoint was based on CIPARS report

^cPercentage resistant

^dValues in the table represent the percentage of isolates showing the defined MIC values

^eVertical lines indicate resistance breakpoints.

Table 3: Multi-drug resistance in sESC-resistant *Escherichia coli* isolates (n=26)

Multi-drug resistance patterns		β-lactamase phenotypes	Number of antimicrobial class*	Frequency	%
1	AmAugCaxCefFox	ESBL, AmpC ^c	3	5	19.20
2	AmCaxCefStrepSulTsTet	ESBL	6	5	19.23
3	AmAugFox ^a	IRT ^b	3	3	11.54
4	AmCaxCefCipGmNalStrepSulTsTet	ESBL	7	2	7.69
5	AmCaxCefCipNalStrepSulTsTet	ESBL	7	2	7.69
6	AmAugCaxCefCipFoxGmKanNalStrepSulTsTet	ESBL, AmpC ^c	8	1	3.85
7	AmAugCaxCefFoxGmSul	ESBL, AmpC ^c	5	1	3.85
8	AmAugCaxCefFoxGmSulTet	ESBL, AmpC ^c	6	1	3.85
9	AmAugCaxCefFoxStrepSulTsTet	ESBL, AmpC ^c	7	1	3.85
10	AmCCaxKanNalStrepSulTsTet	ESBL	8	1	3.85
11	AmCaxCef	ESBL	2	1	3.85
12	AmCaxCefCipGmNalSulTsTet	ESBL	7	1	3.85
13	AmCaxCefNalTet	ESBL	4	1	3.85
14	AmCaxCefStrepSulTet	ESBL	5	1	3.85

Am-Ampicillin, Aug-Amoxicillin-clavulanate, C-Chloramphenicol, Cax-Ceftriaxone, Cef-Ceftiofur, Cip-Ciprofloxacin, Fox-Cefoxitin, Gm-Gentamicin, Kan-Kanamycin, Nal-Nalidixic acid, Strep-Streptomycin, Sul-Sulfisoxazole, Ts-Trimethoprim-Sulfamethoxazole, Tet-Tetracycline

^aNon-ESBL phenotypes

^bSuspected inhibitor resistant *bla*_{TEM}

^cBoth ESBL and AmpC resistance phenotypes

*Isolates showing resistance to at least one antimicrobial in at least 3 antimicrobial classes was considered multi-drug resistant.

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Table 4: Coexistence pattern of β -lactamase resistance genes in sESC-resistant *Escherichia coli* isolates (n=26)

	Patterns	β -lactamase genotypes	Frequency	%
1	bla_{CMY-2}, bla_{TEM}^a	ESBL, AmpC	7	26.9
2	bla_{TEM}, bla_{CTXM-1}	ESBL	5	19.2
3	$bla_{CMY-2}, bla_{TEM}, bla_{CTXM-9}$	ESBL, AmpC	4	15.4
4	$bla_{CMY-2}, bla_{TEM}, bla_{CTXM-1}$	ESBL, AmpC	4	15.4
5	$bla_{TEM}, bla_{SHV}, bla_{CTXM-2}$	ESBL	1	3.85
6	$bla_{CMY-2}, bla_{TEM}, bla_{SHV}$	ESBL, AmpC	1	3.85
7	$bla_{CMY-2}, bla_{TEM}, bla_{CTXM-2}$	ESBL, AmpC	1	3.85
8	bla_{TEM}, bla_{CTXM-9}	ESBL	1	3.85
9	bla_{TEM}	ESBL	1	3.85
10	bla_{CMY-2}	AmpC	1	3.85

^aCould be narrow-spectrum β -lactamase, ESBL or inhibitor resistant bla_{TEM}

Table 5: Distribution of minimum inhibitory concentrations for sQNR-R *E. coli* ($n=21$) recovered from human fecal samples from Prince Edward Island

Antimicrobials	Distribution of MIC (μg/mL)																			
	Range	MIC ₅₀	MIC ₉₀	% R ^c	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Amoxicillin/clavulanate	1 – 32	8	16	4.8								4.8		47.6	42.8	4.8				
Ampicillin	1 – 32	>32	>32	81.0								4.8	9.4	4.8			81.0			
Cefoxitin	0.5 – 32	8	8	4.8								4.8	28.6	57.0	4.8	4.8				
Ceftiofur ^a	0.12 – 8	1	>8	28.6					4.8 ^d	42.8	19.0	4.8			28.6					
Ceftriaxone	0.25 – 64	≤0.25	>64	33.3					66.5				4.8			4.8	4.8	19.1		
Chloramphenicol	2 – 32	8	>32	23.8									9.5	57.2	9.5		23.8			
Ciprofloxacin	0.015 – 4	>4	>4	57.1						14.3	28.6			57.1						
Gentamicin	0.25 – 16	1	>16	14.3						4.8	76.1	4.8				14.3				
Kanamycin	8 – 64	8	>64	33.3										61.8	4.8		4.8	28.6		
Nalidixic acid	0.5 – 32	>32	>32	71.4										9.5	19.1		71.4			
Streptomycin ^b	32 – 64	64	>64	57.1												42.9	9.5	47.6		
Sulfisoxazole	16 – 512	>256	>256	71.4											4.8	9.5	14.3			71.4
Tetracycline	4 – 32	>32	>32	76.2									23.8			4.8	71.4			
Trimethoprim/ Sulfamethoxazole	0.12 – 4	>4	>4	61.8				28.6	4.8			4.8		61.8						

^a CLSI M31–A2,

^bNo Clinical and Laboratory Standards Institute interpretive criteria for *Enterobacteriaceae* were available, breakpoint was based on CIPARS report

^cPercentage resistant

^dValues in the table represent the percentage of isolates showing the defined MIC values

^eVertical lines indicate resistance breakpoints.

1 Table 6: Multi-drug resistance in sQNR-R *Escherichia coli* isolates (n=21)

	Multi-drug resistance patterns	Number of antimicrobial classes resistance*	Frequency
1	AmAugCipKanNalTet	5	1
2	AmCaxCefCipGmKanNalSulTsTet	7	1
3	AmCaxCefCipGmNalStrepSulTsTet	7	1
4	AmCaxCefCipNal	3	1
5	AmCaxCefCipNalStrepSulTsTet	7	1
6	AmCaxCefStrepSulTet	5	1
7	AmCaxCefStrepSulTsTet	6	1
8	AmCCaxKanStrepSulTsTet	7	1
9	AmCCipGmKanNalStrepSulTsTet	7	1
10	AmCCipKanNalStrepSulTet	6	1
11	AmCCipKanNalStrepSulTsTet	7	1
12	AmCipNal	2	1
13	AmCipNalStrep	3	1
14	AmCipNalStrepSulTsTet	6	1
15	AmCKanStrepSulTsTet	6	1
16	AmFoxNal	3	1
17	AmNalStrepSulTsTet	6	1
18	AmNalSulTsTet	5	1
19	CipNalSulTs	3	1
20	SulTsTet	3	1
21	Tet	1	1

2 Am-Ampicillin, Aug-Amoxicillin-clavulanate, C-Chloramphenicol, Cax-Ceftriaxone, Cef-
3 Ceftiofur, Cip-Ciprofloxacin, Fox-Cefoxitin, Gm-Gentamicin, Kan-Kanamycin, Nal-Nalidixic

acid, Strep-Streptomycin, Sul-Sulfisoxazole, Ts-Trimethoprim-Sulfamethoxazole, Tet-Tetracycline

*Isolates showing resistance to at least one antimicrobial in at least 3 antimicrobial classes was considered multi-drug resistant.

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